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A netlike DNA-templated Au nanoconjugate as the matrix of the direct electrochemistry of horseradish peroxidase

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ABSTRACT

Herein, a novel DNA-templated Au nanoparticles (Au-DNA) nanoconjugate was prepared by using the combination of metallization and DNA compaction. The electrostatic interaction between Au(III) and the phosphate backbone of DNA formed the netlike coordination compound of Au(III)-DNA, and then the complex was chemically reduced to form Au nanoparticles in this network-like DNA conformation. The negatively charged nanoconjugate was used as the matrix for immobilization of horseradish peroxidase (HRP). A stable and well-defined redox peaks of HRP were observed on the Au-DNA nanoconjugate modified glassy carbon (GC) electrode, which indicated that the modified enzyme electrode displayed good direct electron transfer behavior and excellent reducing ability toward hydrogen peroxide (H_2O_2) with the apparent Michaelis–Menten constant (K_m) estimated to be 0.147 mM.

1. Introduction

The conjugation of biomaterials and inorganic nanomaterials to yield functional hybrid nanomaterials is a promising route to tailor future sensing and catalytic devices [1–3]. Biological macromolecules are the most appropriate candidate for fabricating functional nanostructured materials [4]. Deoxyribonucleic acid (DNA) seems particularly suitable for this purpose. This molecule is a conductive natural polymer. Its stacked base pairs can be considered as a system of π-electrons connected to transfer electrons so that efficient electron migration within the DNA duplex is possible over a distance up to 40 Å [5]. Moreover, DNA, due to its molecular recognition properties, modularity and high flexibility, has been used as an inexpensive, well characterized, controllable and easily adaptable material to construct defined hybrid nanostructures, especially metal–DNA hybrid nanostructures. Several metals, such as Cu [6], Ag [7], Pd [8] and Au [9,10] have been deposited on specific DNA sequences to produce nanowires expected good electrical conductivity. As we know, the native double-helical DNA provides nucleoside bases and backbone as binding sites for metal ions. In the process of direct binding to DNA, metal complexes can shorten the distances between DNA strands and cause the morphology change [11,12]. In addition, multivalent cation molecules/complexes can transit DNA strands from coil conformation to small compact structure [13]. Therefore, using the properties of DNA for templated fabrication has attracted much attention.

The DNA-templated fabrication could be performed not only on surfaces but also in solutions. We compacted the full-length λ-DNA by using Au(III) complexes and then chemically reduced the Au(III)-coordinated DNA strands to make a network-like hybrid nanostructure Au-DNA in solution. Commonly, Au nanoparticles exhibit strong aggregation tendency in aqueous solution owing to their high specific surface area and surface energy. In our preparation, the netlike structure of DNA could effectively prevent the aggregation of Au nanoparticles. Moreover, this unique structure provides excellent microenvironment for enzymes not only because of good biocompatibility and conductivity of Au nanoparticles [14–16], but also taking advantage of high flexibility and electron transport capacity of DNA.

Here, HRP, as a typical redox enzyme, was immobilized in the resulting Au-DNA host matrix. The Au-DNA/HRP bioconjugate modified glassy carbon (GC) electrode exhibited excellent direct electrochemistry behavior with a formal potential of about −0.31 V (vs. Ag/AgCl) and electrocatalysis of the reduction of H_2O_2. Furthermore, this method provides a novel and simple way to investigate nanoparticles assembly conjugating DNA molecules at low cost in the application in electrochemistry, analytical chemistry and bioelectrochemistry.

2. Experimental sections

2.1. Reagents

λ-DNA with 48502 base pairs (bps) and horseradish peroxidase (HRP, E.C 1.11.1.7, ⩾250 U/mg, from horseradish) were purchased

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from Sigma–Aldrich Biotechnology company (Beijing, China). Other chemicals were purchased from Beijing Chemical Reagent (Beijing, China). All solvents were of analytical grade.

2.2. Preparation of Au-DNA nanoconjugate and chemical modified electrodes

The preparation process of Au-DNA nanoconjugate is shown in Scheme 1. About 10 μL of 780 μM (bps) λ-DNA phosphate buffer solution (PBS) (0.1 M, pH 7.0) was added to 10 μL of 5 mM $HAuCl_4 \cdot 4H_2O$ solution diluted from 10 mM using PBS. The mixture was kept at 4 °C for 2 h with occasional shaking. Subsequently, a small quantity of Au nanoparticle seeds and 20 μL of 78 mM ascorbic acid were added to aforementioned solution. The resulting suspension was kept at 4 °C for 12 h with occasional shaking. Then the mixture was centrifuged at 8000 rpm for 15 min to remove the supernatant. A quantity of 50 μL of deionized water was then added and the hybrid nanomaterials were redispersed. The centrifugation/wash/redispersion cycle was repeated three times to ensure removal of the free DNA and redundant ascorbic acid. The resulting Au-DNA nanoconjugate was then dispersed in 40 μL of pH 7.0 PBS and stored at 4 °C in a refrigerator.

Prior to the surface modification, the GC electrodes (3 mm diameter) were polished with alumina powder (followed by 1.0, 0.3 and 0.05 μm), then rinsed thoroughly with redistilled water, and ultrasonically agitated successively in ethanol and redistilled water, each for 1 min. After the GC electrodes were cleaned and dried in nitrogen, 4.0 μL of the above Au-DNA hybrid nanomaterials suspension and 4.0 μL of 2.0 mg mL^{-1} HRP PBS were mixed together. 8 μL of Au-DNA/HRP dispersion was cast onto the GC electrode surface. As the comparison, Au/HRP/GC electrode (Au nanoparticles were obtained from $HAuCl_4 \cdot 4H_2O$ with ascorbic acid as reducing agent) and DNA/GC electrode were also prepared in the analogous manner, respectively. The as-prepared electrodes were stored at 4 °C in a refrigerator when not in use.

2.3. Instruments

All electrochemical experiments were performed at a CHI 630B electrochemical workstation (CH Instruments Inc., USA) using a conventional three-electrode system with Au-DNA nanoconjugate modified GC electrode, KCl-saturated silver–silver chloride (Ag/AgCl) and a platinum wire as the working, reference and counter electrodes, respectively.

Transmission electron microscopy (TEM) images were obtained with a Hitachi model H-800 (Hitachi, Japan) opened at an accelerating voltage of 100 kV. For TEM measurements, 8 μL of Au-DNA nanoconjugate suspension was dispensed onto a copper grid covered with a continuous carbon film. The sample was dried at room temperature. UV–vis experiments were performed with a UV-2100S spectrophotometer (Shimadzu, Japan).

3. Results and discussion

The Au-DNA hybrid nanostructures were characterized by TEM and UV–vis. As shown in Fig. 1A, the TEM image showed the netlike and porous structures of the Au-DNA nanoconjugate. During the assembly process, DNA acted as the biotemplate and the Au (III) could bond to nucleoside bases. It can be supposed that the compacted structure of λ-DNA was crucial to the formation of Au primary nanoparticles and the hybridization with DNA inhibited the aggregation of Au nanoparticles. In addition, ascorbic acids also

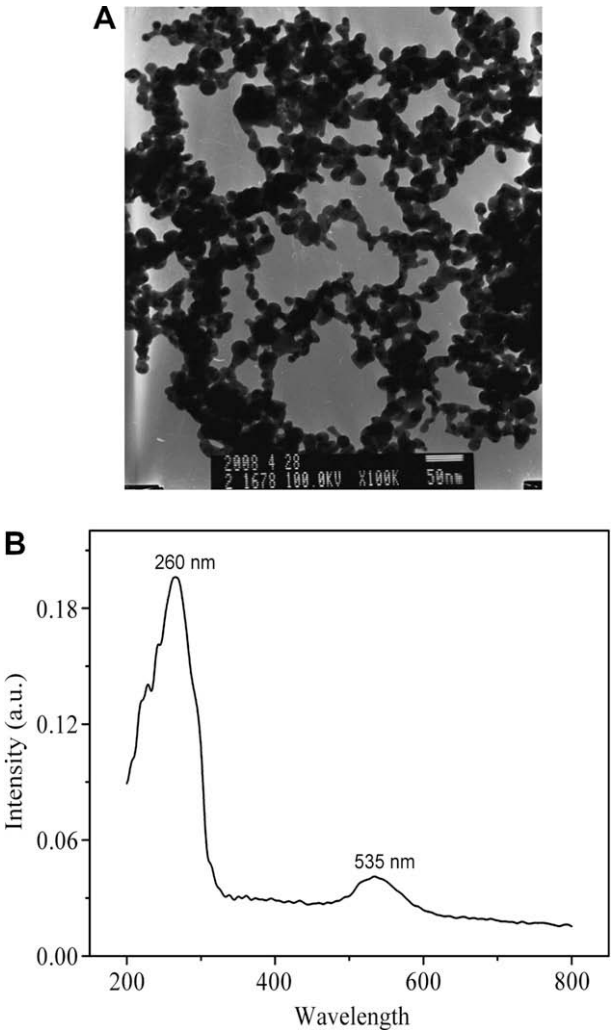
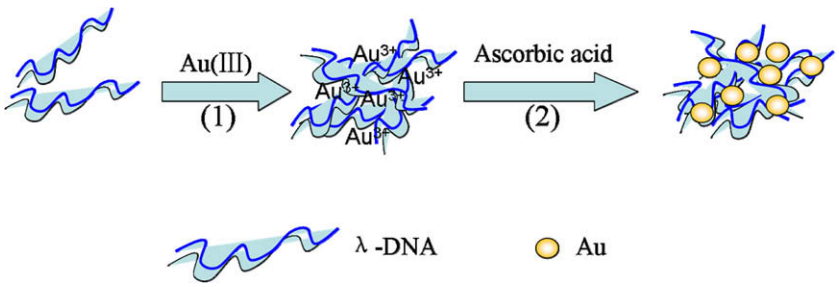


Fig. 1. TEM micrograph (A) and UV–vis spectrum (B) of Au-DNA nanoconjugate.



Scheme 1. Schematic illustration of the preparation procedure of Au-DNA conjugate. (1) The interaction of Au(III) with nucleoside bases of λ-DNA compacts λ-DNA to network-like conformations. (2) The Au(III)-DNA complexes are chemically reduced to form Au nanoparticles by ascorbic acid; as a result, the Au nanoparticles are assembled in a netlike manner on the DNA template.